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Malaria rapid diagnostic tests in travel medicine

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Abstract

Malaria is a serious condition in the non-immune traveller, and prognosis depends on timely diagnosis. Although microscopy remains the cornerstone of diagnosis, malaria rapid diagnostic tests (RDTs) are increasingly used in non-endemic settings. They are easy to use, provide results rapidly and require no specific training and equipment. Reported sensitivities vary between different RDT products but are generally good for *Plasmodium falciparum*, with RDTs detecting the *P. falciparum* antigen histidine-rich protein-2 (PfHRP2) scoring slightly better than *P. falciparum*-lactate dehydrogenase (Pf-pLDH)-detecting RDTs. Sensitivity is lower for *Plasmodium vivax* (66.0 – 88.0%) and poor for *Plasmodium ovale* (5.5 – 86.7%) and *Plasmodium malariae* (21.4 – 45.2%). Rapid diagnostic tests have several other limitations, including persistence of the PfHRP2 antigen, cross-reactions of *P. falciparum* with the non-falciparum test line and *vice versa* and (rare) false-positive reactions due to other infectious agents or immunological factors. False-negative results occur in the case of low parasite densities, prozone effect or *pfhrp2* gene deletions. In addition, errors in interpretation occur, partly due to inadequacies in the instructions for use. Finally, RDTs do not give information about parasite density. In the diagnostic laboratory, RDTs are a valuable adjunct to (but not a replacement for) microscopy for the diagnosis of malaria in the returned traveller.In malaria endemic settings, special groups of travellers (those travelling for long periods, expatriates and short-stay frequent travellers) who are remote from qualified medical services may benefit from self-diagnosis by RDTs, provided they use correctly stored RDT products of proven accuracy, with comprehensive instructions for use and appropriate hands-on training.

Keywords: Diagnosis, malaria, plasmodium, rapid diagnostic test, travel medicine, traveller
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Malaria in the Returned Traveller: a Serious Condition with Difficult Diagnosis

Yearly, approximately 10 000 cases of imported malaria are reported, but the actual number may be as high as 30 000 [1]. Imported malaria is a potentially fatal condition and the outcome depends largely on timely diagnosis and treatment [2]. In malaria non-endemic settings, competence in the microscopic diagnosis of malaria is often lacking because of low exposure to malaria-positive samples [3]. Malaria rapid diagnostic tests (RDTs) may be an alternative: RDTs are simple, hand-held diagnostic devices that offer a quick (within 20 min) diagnosis. Results are visually read as coloured lines on a strip, and no particular expertise is required.

Malaria Rapid Diagnostic Tests: Mechanism, Target Antigens and Formats

Malaria RDTs consist of a nitrocellulose strip mostly embedded in a plastic cassette; occasionally, this strip may present as a dipstick (self-standing strip to be dipped in a tube) or be enclosed in a cardboard format. The mechanism of action is explained in Fig. 1.

The following antigens may be detected: histidine-rich protein-2 (PfHRP2) and *P. falciparum*-specific parasite lactate dehydrogenase (Pf-pLDH) (which are both specific for *P. falciparum*), *P. vivax*-pLDH (Pv-pLDH, specific to *P. vivax*) and pan-pLDH and aldolase (common to all human *Plasmodium* species). Malaria RDTs are categorized as two-, three- or

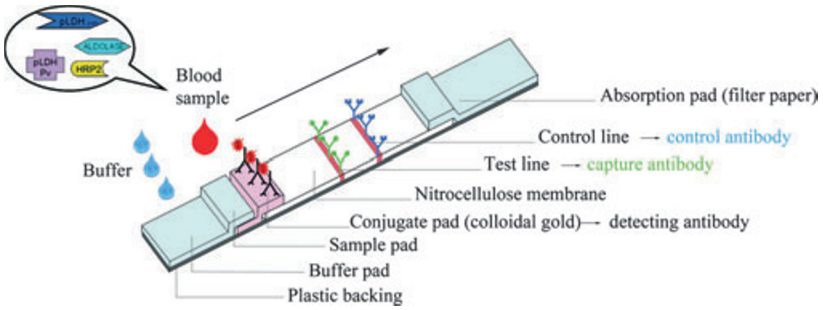

FIG. 1. Schematic drawing of a malaria rapid diagnostic test. Sequence of events when performing an MRDT. Blood and buffer are applied, respectively, to the sample and buffer pad. They are attracted by the capillary action of the absorption pad and start to migrate. First, they pass the conjugate pad, which contains a detection antibody targeting a *Plasmodium* antigen, such as PfHRP2, Pf-pLDH, Pv-pLDH, pan-pLDH or aldolase (for abbreviations see text). This detection antibody is a mouse-antibody that is conjugated to a signal, mostly colloidal gold. If present in the sample, the *Plasmodium* antigen is bound to this detection antibody-conjugate. Next, the antigen-antibody-conjugate complex migrates further until it is bound to the capture antibody, which binds to another site of the *Plasmodium* target antigen. As the capture antibody is applied on a narrow section of the strip, the complex with the conjugated signal will be concentrated and by virtue of the colloidal gold will become visible as a coloured line. The excess of detection antibody-conjugate that was not bound by the antigen and the capture antibody moves further until it is bound to a goat-raised anti-mouse antibody, thereby generating a control line.

four-band products, depending on the number of lines ('bands') that may become visible on the strip, with the control line standing for one band. Two-band RDTs detect a single antigen (mostly PfHRP2), whereas three-band RDTs detect *P. falciparum* (PfHRP2 or Pf-pLDH) and in addition mostly a pan-malaria antigen (pan-pLDH or aldolase); four-band RDTs detect a *P. falciparum*-specific antigen, a pan-malaria antigen and a *P. vivax*-specific antigen (Fig. 2).

Key-points of the Laboratory Diagnosis of Malaria in Returned Travellers

Microscopy represents the cornerstone of malaria diagnosis as it provides all relevant information: confirmation of the diagnosis of malaria; species differentiation (which is important because *P. falciparum* is life-threatening and because treatment is not the

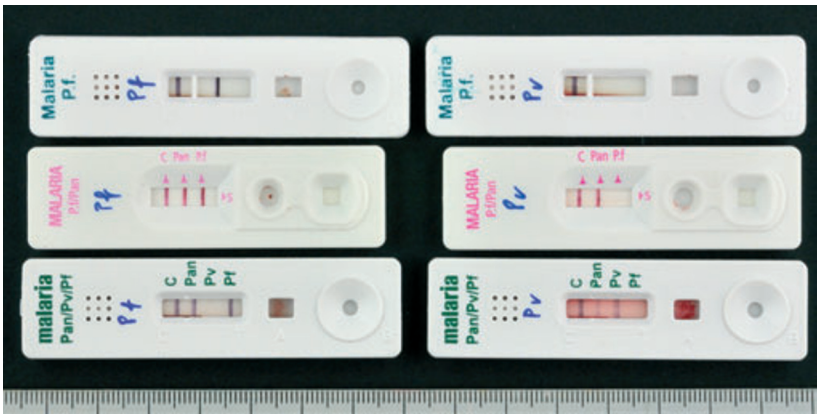

FIG. 2. Two-, three- and four-band malaria RDTs (above, middle and below, respectively) run with a *P. falciparum* sample (left) and a *P. vivax* sample (right). The two-band RDT shows the control line and a *P. falciparum* (PfHRP2)-line for the *P. falciparum* sample and only a control line for the *P. vivax* sample: correct reporting is '*P. falciparum*' and 'no *P. falciparum* detected', respectively. The three-band RDT shows, apart from the control line, PfHRP2 and pan-pLDH test lines for *P. falciparum*: correct reporting is '*P. falciparum*, mixed infection with non-*falciparum* species not excluded'. The *P. vivax* sample shows only a pan-pLDH test line: correct reporting is 'non-*falciparum* species'. For the *P. falciparum* sample, the four-band RDT shows test lines for the pan-pLDH and PfHRP2 test lines: correct reporting is '*P. falciparum*, mixed infection with *P. ovale*/*P. malariae* not excluded'. For the *P. vivax* sample, pan-pLDH and Pv-pLDH test lines are visible: correct reporting is '*P. vivax*, mixed infection with *P. ovale*/*P. malariae* not excluded'.

same for each species [4]); and determination of parasite density, expressed as the number of red blood cells (RBC) infected with asexual parasites (trophozoites and schizontes) per μL of blood. In addition, signs of severity (presence of schizontes and pigment-loaded white blood cells in the case of *P. falciparum*) can be observed. Of note, the detection limit of expert microscopy is close to 50/μL, but for non-experienced microscopists, it is up to ten-fold higher (i.e. 500/μL) [5].

What are the Diagnostic Characteristics of Malaria RDTs in Returned Travellers?

Sensitivity of RDTs for *P. falciparum* diagnosis can reach 100% depending on the product [6–9]. PfHRP2-detecting RDTs generally perform better at low parasite densities compared with Pf-pLDH-detecting RDTs [6,7,9], although this depends on the RDT product [10]. For all RDT products evaluated in our setting (with PCR as the reference method), sensitivity was significantly lower at parasite densities below 100/μL (median 74.1%, range 9.1 – 88.5%) compared with >100/μL (median 94.3%, range 77.4 – 98.1%) [6,7,9–12]. This is of concern as non-immune travellers may be symptomatic below this threshold [13]: at the Institute of Tropical Medicine (ITM), 10% of patients with *P. falciparum* infection presented with parasite density below 100/μL (Table 1).

For *P. vivax* diagnosis, compiled sensitivity of the Binax NOW® kit has been calculated to be 68.9% [13]. At ITM, we observed for other RDT products sensitivities ranging between 66.0% and 88.0%, increasing to 77.4 – 97.2% at parasite densities >500/μL [6,7,9,11,12,14,15]. However, as approximately 10% of patients presenting at ITM had parasite densities <500/μL (Table 1), RDTs will not reliably exclude the presence of *P. vivax*. For *P. ovale* and *P. malariae*, diagnostic sensitivities are much lower, ranging from 5.5% to 86.7% and 21.4% to 45.2%, respectively, with a sharp decline in sensitivity at parasite densities below 500/μL [6,7,9,11,15]. Poor performance in the detection of *P. ovale* and *P. malariae* may partly be explained by the lower affinity of some monoclonal antibodies to these species [16,17].

Plasmodium knowlesi infections are rare in travel medicine. There is no RDT product with a test line specific for *P. knowlesi*, but the species may cross-react with pLDH antibodies specific for *P. falciparum* and *P. vivax* [18]. For six published imported cases of *P. knowlesi*, RDT results were positive in only one patient [19–24].

TABLE 1. Distribution of parasite densities (asexual parasites/μL) per species for the 1495 *Plasmodium*-positive samples submitted to ITM for the period January 2000 to June 2012. Only the first sample per patient was included and samples with exclusively sexual parasites (gametocytes) were excluded

	Single infection, species				
Parasite density (/μL) Number	*P. falciparum*	*P. vivax*	*P. ovale*	*P. malariae*	Mixed infection†
0–100	105	4	16	4	1
101–500	132	18	34	3	5
501–5 000	294	86	63	25	13
5 001–250 000	512	82	14	7	10
>250 000	66	1	0	0	0
Total	1109	191	127	39	29
Cumulative (%)					
≤ 100	9.5	2.1	12.6	10.3	3.4
>100	90.5	97.9	87.4	89.7	96.6
>500	78.6	88.5	60.6	82.1	79.3
>5 000	52.1	43.5	11	17.9	34.5
>250 000	6	0.5	0	0	0

†Mixed infections included *P. falciparum* infection with *P. ovale* (n = 15) or *P. malariae* (n = 12) and *P. malariae* infection with *P. ovale* (n = 1) or *P. vivax* (n = 1).

Does a Positive RDT Result Indicate Malaria Infection?

False-positive RDTs are rare [6,7,9–11,15] but there are no prospective data on their frequency. Over the years 2008–2011 at ITM, we observed false-positive test lines for both a PfHRP2-detecting and a Pf-pLDH-detecting three-band RDT product in 0.4% of samples when using PCR as the reference method (results not published). There was an additional 0.8% of false-positive PfHRP2 lines, which may in part be explained by PfHRP2 persistence after a past *P. falciparum* infection: PfHRP2 can persist in the blood for up to several weeks after successful treatment due to a low clearance [13]. False-positive RDT results in patients with no recent history of malaria have been observed in patients with other infections (e.g. dengue, hepatitis C, toxoplasmosis, tuberculosis and schistosomiasis) or with circulating immunological factors (e.g. rheumatoid factor and anti-nuclear antibodies) [7,25,26].

Malaria RDTs do not Quantify Parasite Density

Rapid diagnostic tests are not designed to give an indication of parasite density. A statistically positive correlation between RDT line intensity and parasite density has been observed but there was overlap between the line intensity categories (faint, weak, medium and strong) [6,7,9]. For example, although strong PfHRP2 line intensities mainly occurred at parasite densities >100/μL, they were also present at lower parasite densities as well as in samples with pure gametocytemia [6,9]. The presence of a single PfHRP2 test line was almost

exclusively found at low (<1 000/μL) parasite densities [6,9,15,27] but it may also indicate PfHRP2 persistence after treated *P. falciparum* infection.

Use of Malaria RDTs in Returned Travellers: Extent and Quality

Two prospective studies performed in routine diagnostic laboratories in the USA concluded that RDTs performed equally as well as or even better than microscopy [28,29]. The use of RDTs in travel medicine is also expanding [3,30], with 80.0% of laboratories declaring that RDTs had improved their malaria diagnosis [3]. A proficiency test performed in Belgium and Luxembourg [3] showed that analytical performance of RDTs was excellent, but errors occurred in interpretation and reporting. Part of these errors was traced to inadequacies in the RDTs' instructions for use and further study revealed multiple shortcomings in packaging, labelling and instructions of many RDT products, including CE-labelled ones [3,31].

Limitations of Malaria RDTs

Rapid diagnostic tests are not foolproof and errors may occur. Apart from the lower sensitivity at low parasite densities discussed above, there is the prozone effect: an absent or faint test line due to an excess of antigens blocking the binding sites of both detection and capture antibodies, hindering binding of the antigen-detection antibody-conjugate complex to the capture antibody, with failure of signal generation. PfHRP2-detecting but not Pf-pLDH-detecting test lines are affected by the prozone effect [32,33]. The prozone effect occurs rarely, but the consequences of a false-negative result are serious, as the diagnosis of malaria (in the case of two-band RDTs) and *P. falciparum* infections (in the case of three-band RDTs) may be missed. Further, although not yet reported in travel medicine, *P. falciparum* isolates lacking the gene encoding for PfHRP2 have been reported in the Peruvian Amazon, and infections by these strains, which may cause symptomatic disease [34], are not picked up by PfHRP2-detecting RDTs [34,35]. Furthermore, cross-reactions of *P. falciparum* samples with the *P. vivax*-pLDH line have been reported [36], as well as cross-reactions of non-*falciparum* samples with the *P. falciparum*-specific test line [6,14,15,34].

What can be Done to Overcome the Limitations of Malaria RDTs?

When the RDT result is negative and there is still suspicion of malaria, repeat testing should be considered (Table 2). As in the case of microscopy, repeat testing does not need to be synchronized with a peak of fever [13] but can be performed after 8–12 h for three consecutive times to rule out malaria [37,38]. Conversely, false-negative results due to the prozone effect cannot be corrected by repeat testing, but when performing microscopy the diagnosis will not be missed as high parasite densities will not be overlooked easily even by non-experienced microscopists. For this reason, as well as for assessing parasite density and signs of severity, we advocate that RDTs should be used in conjunction with microscopy and not as a substitute [3].

What is the Place of Malaria RDTs in the Diagnostic Strategy?

As a complement to microscopy, malaria RDTs can be very useful as they point to the diagnosis of malaria and the

TABLE 2. Role of malaria rapid diagnostic tests in the non-endemic setting

Requirements for malaria diagnosis	Contribution of RDTs	Comments
Timely confirmation or exclusion of the diagnosis of malaria with prompt referral in case of doubt	Considerably helpful in the diagnosis of *P. falciparum* malaria, moderately for *P. vivax* and poorly for *P. ovale* and *P. malariae*	Excellent sensitivity for *P. falciparum* >100 parasites/μL False-negatives for *P. falciparum* at low parasite densities (<100/μL), occasionally above Therefore, repeat after 8–12 h for three consecutive times to rule out malaria
	Do not rule out malaria in a confident way (microscopy needed as well)	Infections caused by *P. falciparum* with $PfHRP_2$ gene deletions will not be picked up by PfHRP2-detecting RDTs Prozone effect is rare but occurs, only PfHRP2- detecting products are affected Moderate (approx. 70%) sensitivity for the diagnosis of *P. vivax* and poor sensitivity (30–50%) for the diagnosis of *P. ovale* and *P. malariae*
Distinction between *P. falciparum* (possible life-threatening) and the non-*falciparum* species	Of considerable help in the identification of *P. falciparum*	Mixed infections are rare but not excluded if *P. falciparum*- and pan-species antigen lines are present
Assessment of parasite densities, in particular recognition of critical values (>2% of red blood cells infected)	Of no help	Line intensities are indicative for parasite density but there is a very large overlap Unique *P. falciparum* line with no pan-species line may point to low parasite density (<1 000/μL)
Recognition of *P. falciparum* stages and hemozoin pigment in white blood cells	Of no help	
Follow-up of treatment (decline of parasite density upon start of therapy)	Of no help	PfHRP2 persists for weeks pLDH, $PfHRP_2$ and aldolase are released by gametocytes

involvement of *P. falciparum* with a detection limit equal to or below that of routine microscopy. Occasionally, the persistence of PfHRP2 may be of help to make an *a posteriori* diagnosis of malaria in a returned traveller who consults after self-administered or empiric treatment for suspected malaria abroad. RDTs have also been studied as a tool for bedside diagnosis ('point of care testing', POC). However, one study showed lower sensitivity of POC compared with laboratory performance and quality and performance of POC testing should be carefully assured [39].

Some Comments about Implementation of RDTs

Most RDTs marketed for malaria diagnosis in returned travellers are three-band RDTs [3], which are of help in differentiating *P. falciparum* from the non-*falciparum* species. Four-band products are used as well [3] but there are few published data and one report mentioned moderate sensitivities, cross-reactions of *Plasmodium* spp. and an excess of lines with faint or weak intensity [15]. Older RDTs (cardboard and hybrid formats) are less easy to use than the more recently released 'one-step' RDTs in cassette format, although differences were not significant when surveyed in laboratory settings [3]. The blood transfer devices supplied with the RDT products (loop, pipette, inverted cup and straw) are small and may be difficult to manipulate [39,40]; this is why we are using a standard transfer pipette. Regarding target antigens for *P. falciparum*, there is a small preference for PfHRP2 over Pf-pLDH-detecting RDT products due to the higher sensitivity at low parasite densities of the former, which is frequently observed in non-endemic settings (Table 1). An overview of the diagnostic performance of most of the commercially available RDT products can be found in reports of the World Health Organization (WHO) and Foundation for Innovative New Diagnostics RDT evaluation rounds [41–43].

Quality Assurance for RDTs in Laboratory use

With regard to ISO 15189 validation and internal quality control, most important is to adequately train laboratory staff, particularly because they will only perform RDTs sporadically. RDTs usually have a shelf life of 18 – 24 months, allowing incidental use. Lot-to-lot variation is not uncommon [7,42–44] and therefore it is of interest to monitor indicators over time, such as (i) invalid test results (they should occur rarely, i.e. <0.5%) [6,7,9–12,14,15,36], (ii) proportions of lines with weak and faint line intensities and (iii) the occurrence of false-positive and false-negative reactions. Validation of implementation should consist of a small panel of samples of *P. falciparum* at high (>1 000/μL) and low (100/μL) parasite densities, as well as a few non-*falciparum* and *Plasmodium*-negative samples. In addition, regular proficiency testing should be organized, and active post-marketing surveillance might trace lot-to-lot variations.

Use of RDTs for Self-diagnosis by Travellers

Travellers and expatriates staying for longer periods in malaria-endemic settings are generally more prone to acquiring malaria infection due to poor adherence to malaria prophylaxis regimens [45,46]. Besides, they are facing particular problems of malaria diagnosis, such as the absence of qualified medical services in remote settings or the tendency to over-diagnose malaria in local laboratories [47–49]. Therefore, the use of self-administration of antimalarials, known as stand-by emergency treatment (SBET), has been advocated for specific groups of travellers, including long-term expatriates and short-stay frequent travellers such as aircrews and business travellers [1,50]. However, malaria recognition based on clinical symptoms is difficult, especially for the non-experienced traveller, and will lead to an overuse of SBET [51]. Combining SBET with a reliable rapid test could prevent its overuse. In the 1990s, use of malaria RDTs was suggested for self-diagnosis of travellers [52].

Several studies on symptomatic travellers performing RDT self-diagnosis revealed major errors in test manipulation (finger prick, application of blood and adherence to the reading time) and interpretation [53–57]. Thereafter RDTs were only recommended for specific situations (e.g. long-term stay, expats, rural areas) after appropriate instructions and training [58]. A more recent evaluation of self-testing among oilfield service employees showed that RDTs can be useful for expatriates when appropriate instructions are received [46].

In recent years, the design and user-friendliness of RDT products have improved and RDTs for self-diagnosis are currently advertised and sold through the internet [59]. We recently evaluated a panel of RDTs marketed for self-diagnosis and found large variations in diagnostic accuracy, as well as poor quality of instructions for use [59]. Before marketing these RDTs for the traveller, improvement of these instructions is needed as well as a comprehensive training programme and follow-up of test results.

In our setting, we offer training on RDT self-diagnosis for travellers referred by physicians during pre-travel consultations. Travellers are provided with a simple layman-directed step-by-step description of the RDT product (www.labquality.be),

which includes comments on limitations of RDTs and on what to do with the test results (e.g. repeat testing in the case of a negative result but persistence of symptoms). The training comprises hands-on practice as well as a photo-based quiz about RDT interpretation. Participants are further invited to email photographs of the RDTs they performed on-site for follow-up and advice. To facilitate finger pricking, we replaced the simple 'plain lancet' provided in the RDT kit with a 'safety lancet' (Sarstedt, Nümbrecht, Germany). Furthermore, we provide extra safety lancets, alcohol wipes and transfer devices, as repeat finger pricking and transfer attempts may be expected. From our training sessions, we learned that repeat hands-on practice is essential for acquiring dexterity in finger pricking and transfer of blood; therefore we recommend RDT self-testing only for those who have been trained or have practised RDTs appropriately; it goes without saying that stressful conditions may decrease the actual performance (Fig. 3). RDTs for self-diagnosis may be useful for the traveller when comprehensive instructions and a training programme are guaranteed, but further study needs to assess their value under field settings.

Conclusion

Malaria RDTs are increasingly used in travel medicine. They contribute to (i) the diagnosis of malaria and (ii) the detection of *P. falciparum*. Their help in the diagnosis of malaria caused by *P. vivax* and *P. ovale*/*P. malariae* is moderate and poor, respectively. Moreover, RDTs do not provide data about parasite density and they are subject to technical and procedural errors. In view of their merits, affordable price and long shelf life, RDTs have a place as a valuable adjunct to microscopy in the laboratory diagnosis of malaria. For special groups of travellers and expatriates not in reach of competent laboratory diagnosis, RDT self-diagnosis is of help, provided there is use of a correctly stored RDT product of proven accuracy and appropriate hands-on training.

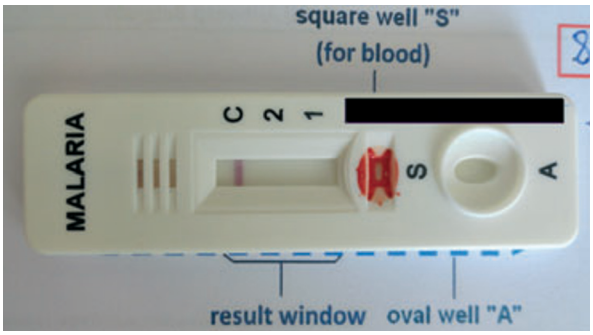

FIG. 3. Picture of a RDT performed by an expatriate and sent by email (permission for publication obtained). A woman performed the RDT on her 9-year-old son in the Ivory Coast and sent the picture by email. She had attended the RDT training 3 months earlier. The cassette is placed on the printed outlines of the layman-directed Standard Operating Procedure (www.labquality.be). From the picture it is clear that the user had difficulties applying the blood into the sample well (S), as much blood sticks to the plastic casing. Applying a too low volume of blood may result in false-negative RDT results. The visible control line (C) only testifies that migration of the colloidal gold complex occurred, but not that the correct sample volume has been applied.

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